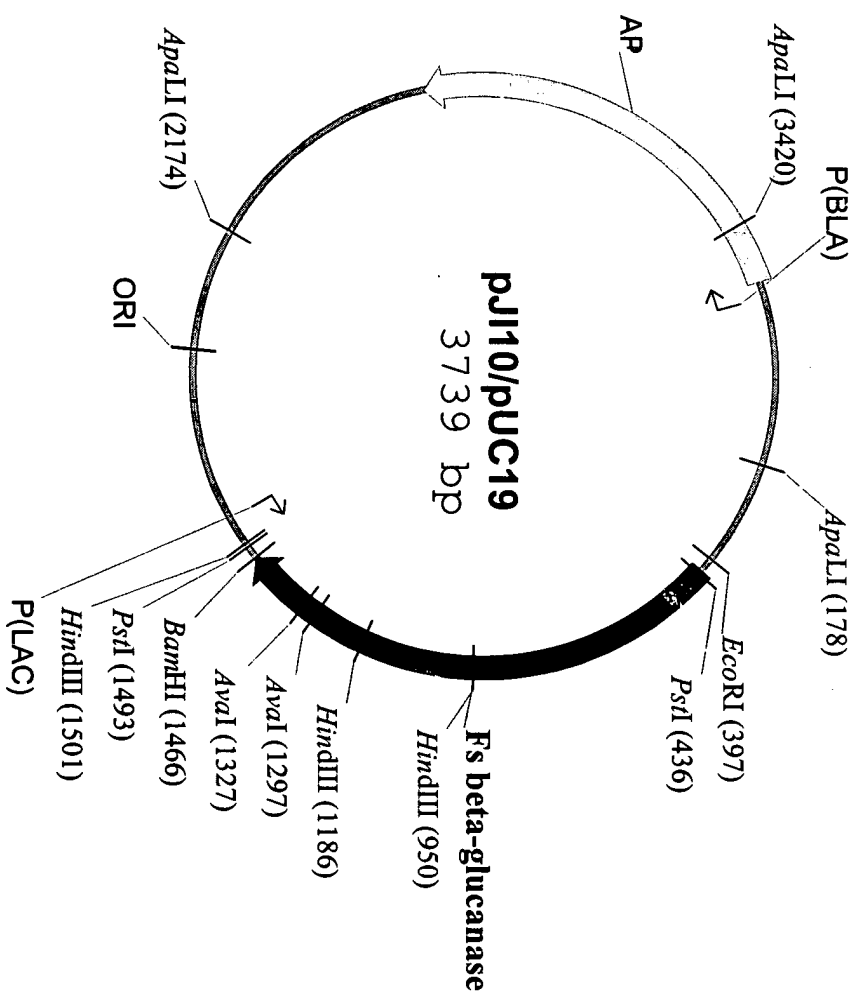


Fig. 1



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Fig. 2 The amino acid sequence in Fig. 1's SEQ ID NO: 1  
 the DNA sequence in Fig. 2 is SEQ ID NO: 4

ATGGTTAGCGCAAAGGATTTTAGCGGTGCCGAACCTACACGTTAGAAGAAGTTCAGTAC  
 M V S A K D F S G A E L Y T L E E V Q Y 20

GGTAAGTTTGAAGCCCGTATGAAGATGGCAGCCGCATCGGGAACAGTCAGTTCCATGTTC  
 G K F E A R M K M A A A S G T V S S M F 40

CTCTACCAGAATGGTTCGAAATCGCCGATGGAAGGCCCTGGGTAGAAGTGGATATTGAA  
 L Y Q N G S E I A D G R P W V E V D I E 60

GTTCTCGGCAAGAATCCGGGCAGTTTCCAGTCCAACATCATTACCGGTAAGGCCGGCGCA  
 V L G K N P G S F Q S N I I T G K A G A 80

CAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGCCGATCAGGCTTTCACACC  
 Q K T S E K H H A V S P A A D Q A F H T 100

TACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAGGAAGTCCGC  
 Y G L E W T P N Y V R W T V D G Q E V R 120

AAGACGGAAGGTGGCCAGGTTTCCAACCTTGACAGGTACACAGGGACTCCGTTTAAACCTT  
 K T E G G Q V S N L T G T Q G L R F N L 140

TGGTCGTCTGAGAGTGCGGCTTGGGTGGCCAGTTCGATGAATCAAAGCTTCCGCTTTTC  
 W S S E S A A W V G Q F D E S K L P L F 160

CAGTTCATCAACTGGGTCAAGGTTTATAAGTATACGCCGGGCCAGGGCGAAGGCCGCAGC  
 Q F I N W V K V Y K Y T P G Q G E G G S 180

GACTTTACGCTTGACTGGACCGACAATTTTGACACGTTTGATGGCTCCCGCTGGGGCAAG  
 D F T L D W T D N F D T F D G S R W G K 200

GGTGACTGGACATTTGACGGTAACCGTGTCGACCTCACCGACAAGAACATCTACTCCAGA  
 G D W T F D G N R V D L T D K N I Y S R 220

GATGGCATGTTGATCCTCGCCCTCACCCGCAAAGGTCAGGAAAGCTTCAACGGCCAGGTT  
 D G M L I L A L T R K G Q E S F N G Q V 240

CCGAGAGATGACGAACCTGCTCCG  
 P R D D E P A P 248

Fig. 3

The amino acid sequence in Fig. 3 is SEQ ID NO: 2 <sup>Fig. 3</sup> <sup>Shyur et al</sup>

The DNA sequence in Fig. 3 is SEQ ID NO: 5

ATGGTTAGCGCAAAGGATTTTAGCGGTGCCGAAGCTCTACAGTTAGAAGAAGTTCAGTAC  
M V S A K D F S G A E L Y T L E E V Q Y 20

GGTAAGTTTGAAGCCCGTATGAAGATGGCAGCCGCATCGGGAACAGTCAGTTCCATGTTT  
G K F E A R M K M A A A S G T V S S M F 40

CTCTACCAGAATGGTTCCGAAATCGCCGATGGAAGGCCCTGGGTAGAAGTGGATATTGAA  
L Y Q N G S E I A D G R P W V E V D I E 60

GTTCTCGGCAAGAATCCGGGCAGTTTCCAGTCCAACATCATTACCGGTAAGGCCGGCGCA  
V L G K N P G S F Q S N I I T G K A G A 80

CAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGCCGATCAGGCTTTCCACACC  
Q K T S E K H H A V S P A A D Q A F H T 100

TACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAGGAAGTCCGC  
Y G L E W T P N Y V R W T V D G Q E V R 120

AAGACGGAAGGTGGCCAGGTTTCCAAGTTGACAGGTACACAGGGACTCCGTTTTAACCTT  
K T E G G Q V S N L T G T Q G L R F N L 140

TGGTCGTCTGAGAGTGGCGCTTGGGTTGGCCAGTTCGATGAATCAAAGCTTCCGCTTTTC  
W S S E S A A W V G Q F D E S K L P L F 160

CAGTTCATCAACTGGGTCAAGGTTTATAAGTATACGCCGGGCCAGGGCGAAGGCCGCAGC  
Q F I N W V K V Y K Y T P G Q G E G G S 180

GACTTTACGCTTGACTGGACCGACAATTTTGACAGTTTGATGGCTCCCGCTGGGGCAAG  
D F T L D W T D N F D T F D G S R W G K 200

GGTGACTGGACATTTGACGGTAACCGTGTGACCTCACCGACAAGAACATCTACTCCAGA  
G D W T F D G N R V D L T D K N I Y S R 220

GATGGCATGTTGATCCTCGCCCTCACCCGCAAAGGTCAGGAAAGCTTCAACGGCCAGGTT  
D G M L I L A L T R K G Q E S F N G Q V 240

CCGAGAGATGACGAACCTGCTCCGTAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTC  
P R D D E P A P N S S S V D K L A A A L 260

GAGCACCACCACCACCACCTGA  
E H H H H H H \*

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Fig. 4.

Table 1. Comparison of kinetic properties of *F. succinogenes* and *B. subtilis* 1,3-1,4-β-D-glucanases

Enzyme	Specific activity (U/mg)	$k_{cat}$ ( $s^{-1}$ )	Opt. Temperature (°C)	Opt. pH
Wild-type	2065 ± 82	1296 ± 51	50 (at pH 6.0)	6.0-8.0
TG-Glucanase	7980 ± 341	3695 ± 158	50 (at pH 6.0)	6.0-8.0
PCR-TF-Glucanase	7833 ± 334	3911 ± 166	50 (at pH 6.0)	6.0-8.0
Lichanase (Megazyme)	118 <sup>a</sup>	47.2 <sup>a</sup>	60 (at pH 6.5) <sup>a</sup>	6.5-7.0 <sup>a</sup>
	82.6 ± 0.96	33.0 ± 0.38	55 (at pH 7.0)	

The kinetics was performed with lichenan (6mg/mL) as substrate in 50 mM citrate buffer (pH 6.0) or in 50mM phosphate buffer (pH 7.0), and at optimum temperature as indicated.

<sup>a</sup>. Data was taken from *Megazyme* instruction brochure of lichenase. The kinetics was done with barley β-glucan (5mg/mL) as substrate.

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Fig. 5

Table 3. Reactivation of PCR-TF-glucanase at 25 °C after heat treatment

Heat treatment	Recovery time (min)	Relative activity (%)
90 °C, 10 min	10	68
	20	81
90 °C, 30 min	10	61
	20	67
100 °C, 10 min	10	68
	20	72
100 °C, 30 min	10	55
	20	56

Fig. 6a The no acid sequence in Fig. 6 is SEQ ID NO: 3  
(part a) The DNA sequence in Fig. 6 is SEQ ID NO: 6

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ATGAACATCAAGAAACTGCAGTCAAGAGCGCTCTCGCCGTAGCAGCCGCAGCAGCAGCC
M N I K K T A V K S A L A V A A A A A A 20

CTCACCACCAATGTTAGCGCAAAGGATTTTAGCGGTGCCGAAGTCTACACGTTAGAAGAA
L T T N V S A K D F S G A E L Y T L E E 40

GTTACGTACGGTAAGTTTGAAGCCCGTATGAAGATGGCAGCCGCATCGGGAACAGTCAGT
V Q Y G K F E A R M K M A A A S G T V S 60

TCCATGTTCTCTACCAGAATGGTTCCGAAATCGCCGATGGAAGGCCCTGGGTAGAAGTG
S M F L Y Q N G S E I A D G R P W V E V 80

GATATTGAAGTTCTCGGCAAGAATCCGGGCAGTTTCCAGTCCAACATCATTACCGGTAAG
D I E V L G K N P G S F Q S N I I T G K 100

GCCGGCGCACAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGCCGATCAGGCT
A G A Q K T S E K H H A V S P A A D Q A 120

TTCCACACCTACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAG
F H T Y G L E W T P N Y V R W T V D G Q 140

GAAGTCCGCAAGACGGAAGGTGGCCAGGTTTCCAAGTACAGGTACACAGGGACTCCGT
E V R K T E G G Q V S N L T G T Q G L R 160

TTTAACCTTTGGTTCGTCTGAGAGTGCGGCTTGGGTTGGCCAGTTCGATGAATCAAAGCTT
F N L W S S E S A A W V G Q F D E S K L 180

CCGCTTTTCCAGTTCATCAACTGGGTCAAGGTTTATAAGTATACGCCGGGCCAGGGCGAA
P L F Q F I N W V K V Y K Y T P G Q G E 200

GGCGGCAGCGACTTTACGCTTGACTGGACCGACAATTTTGACACGTTTGATGGCTCCCGC
G G S D F T L D W T D N F D T F D G S R 220

TGGGGCAAGGGTGACTGGACATTTGACGGTAACCGTGTGACCTCACCGACAAGAATC
W G K G D W T F D G N R V D L T D K N I 240

TACTCCAGAGATGGCATGTTGATCCTCGCCCTCACCCGCAAAGGTCAGGAAAGCTTCAAC
Y S R D G M L I L A L T R K G Q E S F N 260

GGCCAGGTTCCGAGAGATGACGAACCTGCTCCGCAATCTTCTAGCAGCGCTCCGGCATCT
G Q V P R D D E P A P Q S S S S A P A S 280

TCTAGCAGTGTTCCGGCAAGCTCCTCTAGCGTCCCTGCCTCCTCGAGCAGCGCATTTGTT
S S S V P A S S S S V P A S S S S A F V 300

CCGCCGAGCTCCTCGAGCGCCACAAACGCAATCCACGGAATGCGCACAACTCCGGCAGTT

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Fig. 6  
(part b)

P P S S S S A T N A I H G M R T T P A V 320  
GCAAAGGAACACCGCAATCTCGTGAACGCCAAGGGTGCCAAGGTGAACCCGAATGGCCAC  
A K E H R N L V N A K G A K V N P N G H 340  
AAGCGTTATCGCGTGAAC TTTGAACACTAA  
K R Y R V N F E H \* 349